

WILSON et al.
Serial No. 09/787,633

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elongated (at least x3 length of host cells) but they survived for only a few generations.
PCR from these colonies to determine if a "knock—out" had occurred was
unsuccessful.--

Insert the attached [✓]Sequence Listing in place of that which was filed with the
Preliminary Amendment on March 21, 2001.

REMARKS


Reconsideration is requested.

The present Amendment is submitted in response to the communication received
with the Office Action of January 14, 2002. A separate Response will be filed to the
Office Action (Paper No. 11).

The attached paper and computer-readable copies of the Sequence Listing are
the same. No new matter has been added. A separate Statement to this effect is
attached.

Respectfully submitted,

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MARKED UP SPECIFICATION

Pages 19 and 20, delete the paragraph spanning page 19, line 34 through page 20, line 14, and insert the following therefor:

--A central segment of the wild type gene (slr0074) from *Synechocystis* sp., strain pCC6803, (delineated by two *Hind*III sites ~1.0 kb apart) was amplified from genomic DNA by PCR using two oligonucleotide primers based on the known sequence (Accession no. S76598): 5' CTC CGC CCC TAA GCA AAG TAA GAA A (SEQ ID NO:4) and 5' TGA TCC TCG CCA ATT TTG GAA GTG GA (SEQ ID NO:5). The amplified fragment was restricted with *Hind*III and cloned into pUC9 to give pUC9/slr . The sequence of both ends of the insert was confirmed in recombinants grown in Epicurian SURE cells. To disrupt the gene, a kanamycin resistance gene (*k'*) isolated from a modified bluescript vector (pBSHdSpl, was blunt—ended with the Klenow fragment and cloned into a unique *Xba*I site in the *ycf* 24 insert to give pUC9/slr/*k'*. Recombinants in XLI-blue MRF' cells were selected using kanamycin. Disruption of the *ycf* 24 insert was confirmed by sequencing the junction regions.--

Pages 20 and 21, delete the paragraph spanning page 20, line 32 through page 21, line 19, and insert the following therefor:

-- Disruption of *ycf* 24 in *E. coli* was carried out along similar lines, but in this case disruption was with the *aadA* gene for streptomycin resistance (*S'*). *ycf* 24 from *E. coli* was amplified using primers based on the accession no. D90811 with added terminal restriction sites: 5' GAG CTC GGA ATT CGC ATG TGG CTG TGG CGA AAG (SEQ ID NO:6) and 3' GAG CTC GGG ATC CTT ATC CGA CGC TGT GTT CAA G

(SEQ ID NO:7). The *E.coli aadA* gene was introduced at a *BsgI* restriction site near the centre of *ycf 24* and cloned into the bluescript vector pBSKS⁺. The construct was linearized for transformation and to allow homologous recombination in *E. coli* LE392 host cells. The linearized recombinant plasmid pBSKS⁺/*ycf24*/S^r was transfected into *E. coli* by heat shock but no homologous recombinants were found from the primary normal size colonies; a few antibiotic resistant colonies were recovered that carried circular episomes likely to have arisen from re-ligation of the construct. After 2 days incubation at 37⁰C small colonies were observed. Cells from these were found to be elongated (at least x3 length of host cells) but they survived for only a few generations. PCR from these colonies to determine if a "knock—out" had occurred was unsuccessful.--